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13. ABSTRACT *(Maximum 200 words)*

We have developed a system of chemically synthesized copolypeptides and chemical oxidants that provide a convenient, easily produced alternative to marine adhesive proteins for underwater adhesion. Our recent mechanistic studies have revealed many interesting features of these copolymers relating to their effectiveness as underwater adhesives. First, that **o**-quinone is the **only** chemical functionality required for crosslinking opens up many possibilities for the design of moisture-resistant adhesives for specialized applications. Second, our discovery that catechol, and not **o**-quinone, is primarily responsible for adhesion has important ramifications in practical application of these materials. If the polymers is oxidized too rapidly, there will be poor adhesion since all the catechol has been consumed: a phenomenon we have observed and which had not previously been considered. Overall, our mechanistic studies have given us tremendous insight into possible ways to imporve these materials.

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FINAL REPORT

GRANT #: N00014-96-1-0729

PRINCIPAL INVESTIGATOR: Dr. Timothy J. Deming

INSTITUTION: University of California at Santa Barbara

GRANT TITLE: High Performance Underwater Adhesives: Synthetic Analogs of Marine Mussel Cement Proteins.

AWARD PERIOD: 1 June 1996 - 31 May 1999

OBJECTIVE: To chemically synthesize and characterize polymeric materials possessing the characteristics of marine adhesive proteins, which can be prepared in large scale with consistent composition and control over their physical properties. To understand and capitalize on the mechanisms employed by marine adhesives for development of moisture-resistant adhesives.

APPROACH: We have chemically synthesized copolypeptides that contain side-chain functional groups (e.g. catechol and primary amine) that are present in natural mussel adhesive proteins. These random copolymers display moisture-resistant adhesive properties which rival those of mussel adhesive proteins. Furthermore, we have also prepared a variety of copolypeptides containing different functional side chains (e.g. hydrophilic, hydrophobic, beta-sheet forming, alpha-helix forming) to probe the effects of the composition and sequence of these groups on adhesive and crosslinking behavior. Model studies with small molecules have also been undertaken to gain mechanistic insight into the roles of the putative active protein components in crosslink formation and adhesion to a variety of substrates. This information was used to improve the properties (resistance to swelling, substrate specificity, bulk strength, etc.) of our adhesives.

ACCOMPLISHMENTS: Using the known compositions of many natural adhesive proteins, we have prepared statistically random copolypeptides containing the amino acid residues thought to be active in adhesive formation. We have synthesized our copolymers by polymerization of select α-amino acid N-carboxy anhydrides (NCAs) using strong base initiators. We initially prepared binary copolypeptides of L-lysine and L-DOPA containing compositions of the two monomers ranging from 0 to 50 mole percent DOPA. These polymers were found to be soluble in aqueous buffers over a wide pH range (*ca.* 2 -12). We have also prepared ternary copolypeptides containing the amino acids L-serine and L-alanine in addition to DOPA and lysine. The serine and alanine residues were inserted to act as spacers for the functional groups thought to be active in adhesive formation. To analyze crosslinking ability, we monitored the rheological behavior of aqueous solutions of the copolymers as functions of monomer composition, pH, and oxidizing agent. The oxidizing agents utilized were mushroom tyrosinase, O_2, KIO_4, H_2O_2, and $Fe(H_2O)_6^{3+}$. The oxidizing agents were expected to convert the catechol functionalities of the DOPA residues into *ortho*-quinone units which are believed to be responsible for a variety of crosslinking reactions and/or adhesion. By variation of the oxidizing agent and pH, we were able to obtain systems where gel

formation times could be adjusted from seconds to hours. In conjunction with these experiments, we have conducted adhesion measurements to correlate gel formation with adhesive strength. Initial measurements using steel and aluminum adherends have shown that molecular oxygen is a very effective oxidizing agent for formation of strong bonds, where cure time can be adjusted by variation of solution pH. In terms of polymer composition, the strength of adhesive bonds was found to increase proportionally with the amount of DOPA in the copolymer. The oxidation, adhesive-forming capability, and crosslinking behavior of these copolymers were studied under a variety of environmental conditions including variation of copolymer composition, molecular weight, cure time, temperature, and oxidant. The results of these studies are described in detail in publication #1. In short, we were able to determine the optimal conditions for curing and application of our simple copolymers, which displayed moisture-resistant adhesive properties comparable to mussel adhesive proteins.

In addition to this work, we have made substantial progress in elucidating the roles played by the different components of mussel adhesives. Since our copolymers are functionally simple, compared to the natural proteins, we were able to model the reactivity of our copolymers with small model compounds. We have found, in both model compounds and the copolymers, that crosslinking occurs solely through homocoupling of *o*-quinone groups, and not through coupling of *o*-quinone with the primary amino groups of lysine residues (Publication # 4). Hence, lysine (whose protonated amino groups result in a cationic copolymer) is only responsible for water solubility and can be replaced with other ionic groups (i.e. anionic carboxylate) without strongly adversely affecting adhesive properties. In addition, by examining the adhesive capabilities of our copolymers in the strict *absence* of oxidant, we were able to probe the relative roles of catechol and *o*-quinone in adhesion to a variety of substrates. These studies unequivocally showed that catechol is the dominant functionality in the adhesion process: thus oxidation is only necessary for crosslinking, but not adhesion.

Based on these data, we have synthesized new copolypeptides containing a variety of amino acid components to replace the lysine component of our original synthetic polypeptide adhesives. These experiments were based on our discovery that the catechol functionality of DOPA was the only functional component necessary for realizing moisture-resistant adhesion. Full analysis of the properties of these copolymers has not yet been completed.

CONCLUSIONS: Our system of chemically synthesized copolymers and chemical oxidants provides a convenient, easily produced alternative to marine adhesive proteins for underwater adhesion. Our recent mechanistic studies have revealed many interesting features of these copolymers relating to their effectiveness as underwater adhesives. First, that *o*-quinone is the *only* chemical functionality required for crosslinking opens up many possibilities for the design of moisture-resistant adhesives for specialized applications. For example, the ability to incorporate either positively or negatively charged groups into the adhesives allows for selective binding to charged surfaces. Second, our discovery that catechol, and not *o*-quinone, is primarily responsible for adhesion has important ramifications in practical application of these materials. If the polymer is oxidized too rapidly, there will be poor adhesion since all the catechol has been consumed: a phenomenon we have observed and which had not previously been considered. Overall, our mechanistic studies have given us tremendous insight into possible ways to improve these materials.

SIGNIFICANCE: Our results are important in that we have shown that the remarkable moisture-resistant adhesive properties of marine proteins can be reproduced in readily prepared synthetic polymers. This advance for the first time allows for the large-scale preparation of moisture-resistant adhesives, a major limitation in the past due to problems in isolation of sizable quantities of adhesive protein from biological sources. Our studies on the mechanisms of mussel adhesives have considerable potential to enhance the properties of synthetic adhesives. We have shown that catechol is the only functionality required for effective moisture-resistant adhesion. This result implies that most any synthetic polymer, when functionalized with catechol groups, should display enhanced moisture-resistant adhesion. Using inexpensive commodity polymers, large-volume commodity underwater adhesives could be developed.

PATENT INFORMATION: "Synthesis and Crosslinking of Catechol Containing Copolypeptides" U.S. Patent application filed 3/19/99 by the University of California.

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2) Yu, M.; Deming, T. J. (1998) "Synthetic Polypeptide Mimics of Marine Adhesives". Macromolecules 31: 739-4745.

3) Deming, T. J. (1999) "Mussel Byssus and Biomolecular Materials". Curr. Opn. Chem. Biol. 3: 100-105.

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Synthetic Analogs of Marine Mussel Cement Proteins

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Introduction

Surgical tissue adhesives provide an alternative to suturing, packing, or stapling planes of tissue together. Use of adhesives for wound closure is desirable since the adhesives can be very fast acting and assure complete closure. Polymers of α-cyanoacrylates have been investigated clinically as potential tissue adhesives since they are able to adhere to and cement together moist living tissues.[1] These adhesive polymers are fast acting, hemostatic and have also been found to be bacteriostatic.[1] However, the polymers can degrade *in vivo* to toxic and inflammatory byproducts and may have deleterious long-term health effects.[2] These factors have resulted in the recommendation that these adhesives be used clinically only as a lifesaving procedure in emergency situations. As a consequence of this, many other materials and techniques have been studied for use as superior tissue adhesives; these include fibrin-based sealants, laser solders, and marine adhesive proteins.

The prolific adhesive capabilities of many marine organisms are well known. Each year considerable time and money is spent worldwide on the removal of barnacles from fouled vessels and other man-made structures exposed to the oceans.[3] The adhesives are remarkable in that they can function over wide temperature ranges (-40 to 40 °C), fluctuating salinities, humidities, and in the tides, waves and currents of marine environments.[4] These glues are able to form a permanent bond in a few seconds to a wide variety of substances with complex and often irregular surface coatings. In contrast, the success of synthetic adhesives in wet environments requires carefully cleaned adherends which often must also be chemically treated and/or partially dried.[5] Furthermore, preliminary immunological studies on marine adhesive proteins revealed that they are very poor antigens and thus excellent candidates for *in vivo* applications.[6] Knowledge and understanding of the materials and mechanisms used by mussels and barnacles to adhere to underwater surfaces would be valuable for the design and synthesis of superior adhesives for use as tissue adhesives and dental cements.

The adhesive proteins isolated from mussels (e.g. *Mytilus edulis*, Figure 1),[4] and recombinant proteins of similar sequences expressed in microbes,[7] have been purified and examined for use as tissue adhesives. Preliminary experiments indicated that these proteins are very effective for formation of adhesive bonds to animal tissues and also exhibit low immunogenicity.[8] The major drawbacks with these materials are that (i) their mechanisms of action are poorly understood, (ii) the essential requirements for good adhesion and crosslinking are unknown, and (iii) these proteins cannot be produced inexpensively or in the large quantities necessary for successful commercial application.[4,8]

Organism	MW	Consensus peptide
Phragmatopoma californica	35 kD	ValGlyGlyDOPAGlyDOPAGlyAlaLys
Mytilus edulis	130 kD	AlaLysProSerTyrdiHypHypThrDOPALys
Geukensia demissa	130 kD	ThrGlyDOPAGlyProGlyDOPALys
Aulacomya ater	130 kD	AlaGlyDOPAGlyGlyLeuLys
Dreissena polymorpha	76kD	GlyProDOPAValProAspGlyProTyrAspLys
Brachidontes exustus	105kD	GlyLysProSerProDOPAAspProGlyDOPALys

Figure 1 Sequences of some naturally occurring marine adhesive protein repeats

The focus of our work has been to avoid the complexities associated with synthesis and analysis of natural proteins by use of peptides and polymers which contain only the minimal functional groups necessary for cement formation. Concurrent polymer synthesis coupled with characterization should then lead to a rapid convergence of crosslink mechanism determination and discovery of the essential adhesive components. Hence, we will be able to test the hypothesis of whether or not the sequences and structures of natural adhesive proteins play dominant roles in governing their ultimate properties. Simplified materials will also allow the design and synthesis of marketable adhesives prepared through low cost, high volume polymerization techniques.

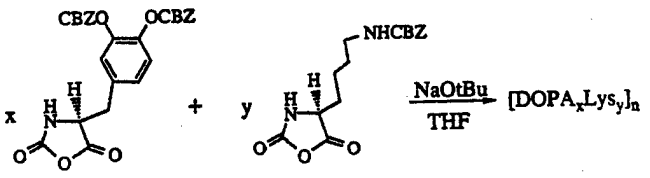
Experimental

Preparation of the α-amino acid-N-carboxyanhydrides of di-(O-CBZ)-L-dihydroxyphenylalanine (di-(O-CBZ)-L-DOPA), ε-(N-CBZ)-L-Lysine, (OBz)-L-Serine, and L-Alanine were performed either in a N_2 filled dry box or on a Schlenk line according to literature procedures.[9] These monomers were recrystallized until free of halide and were stored at -10 °C under vacuum until used. Polymerizations were run in THF under N_2 atmosphere in sealed vessels using NaO*t*Bu initiator at 25 °C for 5 days. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on a Spectra Physics Isochrom liquid chromatograph pump equipped with a Wyatt DAWN DSP light scattering detector and Wyatt Optilab DSP. Separations were effected by 10^5Å, 10^4Å, and 10^3Å Phenomenex 5μm columns using 0.10 M LiBr in DMF as eluent. NMR spectra were recorded on Bruker AMX 500MHz and Varian Gemini 200MHz spectrometers.

Results and Discussion

This project is focused on elucidating the adhesion and crosslinking mechanisms of marine adhesive proteins. This information will then be utilized to design and synthesize simplified adhesive polymers which incorporate the essential functional components of the marine proteins. One key hypothesis we wish to test is whether the specific amino acid sequences found in natural adhesive proteins are necessary for effective adhesive formation. In other words, will a *random* sequence of the same amino acids found in a sequence-specific protein display adhesive properties similar to the natural material? This question is important when considering the low cost and ease of preparation of random versus sequence-specific copolypeptides. Inexpensive, mass produced adhesive polymers based on random copolypeptides will have the potential to become commercially viable tissue adhesive materials.

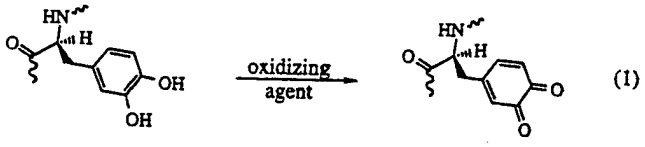
Using the known compositions of many natural adhesive proteins, we have prepared sequentially random copolypeptides through copolymerization of a few select α-amino acid N-carboxy anhydrides (NCAs) (Scheme 1).[9] NCAs are readily prepared from amino acids by phosgenation and can be polymerized into high molecular weight polypeptides via successive ring opening addition reactions which liberate carbon dioxide. A similar approach for making adhesive polymers using NCA polymerizations has been reported by Yamamoto and coworkers.[10] They have prepared random copolypeptides containing the amino acids Glutamic acid, DOPA, Tyrosine, and Lysine, as well as a complex mixture of 17 different NCAs.[11] Their work was focused on conformational analyses of the polymers and there was no attempt to determine the amount of moisture resistance in adhesion measurements. Furthermore, the adhesive roles of the catechol versus the oxidized *o*-quinone functionalities of DOPA were not separately evaluated.



Scheme 1 One step preparation of adhesive copolypeptides using NCA monomers

We initially prepared very simple copolypeptides containing Lysine and DOPA containing different compositions of the two monomers. These polymers were found to be soluble in aqueous buffers over wide pH ranges (*ca* 2 -12). We then evaluated the ability of these polymer solutions to form crosslinked gels as functions of monomer composition, pH, and oxidizing agent. The oxidizing agents utilized were O_2, KIO_4, and $Fe(H_2O)_6^{3+}$. The oxidizing agents were expected to convert the catechol functionalities of the DOPA residues into *o*-quinone units which are believed to be responsible for a variety of crosslinking

reactions (eq 1).[4] By variation of the oxidizing agent and pH, we were able to obtain systems where gellation times could be adjusted from seconds to hours. In conjunction with these experiments, we are conducting adhesion measurements to correlate gellation with adhesive forming ability.



(1)

Acknowledgment

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Synthetic Polypeptide Mimics of Marine Adhesives

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Synthetic Polypeptide Mimics of Marine Adhesives

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ABSTRACT: Water soluble copolypeptides containing L-dihydroxyphenylalanine (DOPA) and L-lysine were prepared by ring-opening polymerization of α-amino acid *N*-carboxyanhydride (NCA) monomers. We have prepared a range of different copolymers to probe the effects of functional group composition on adhesive and cross-linking behavior. Aqueous solutions of these copolymers, when mixed with a suitable oxidizing agent (e.g., O_2, mushroom tyrosinase, Fe^{3+}, H_2O_2, or IO_4^-), formed cross-linked networks that were found to form moisture-resistant adhesive bonds to a variety of substrates (e.g., aluminum, steel, glass, and plastics). It was found that successful adhesive formation was dependent on oxidation conditions, with chemical oxidants giving the best results. Optimized systems were found to form adhesive bonds that rival in strength those formed by natural marine adhesive proteins. Our synthetic systems are readily prepared in large quantities and require no enzymes or other biological components.

Introduction

The prolific adhesive capabilities of many marine organisms are well-known. Each year a considerable effort is spent worldwide on the removal of barnacles and mussels from fouled vessels and other man-made structures exposed to the oceans.[1] The adhesives are remarkable in that they function over wide temperature ranges (−40 to 40 °C), fluctuating salinities, and humidities and in the tides, waves, and currents of marine environments.[2] These glues are able to form permanent bonds in a few seconds to a wide variety of substrates with complex and often irregular surface coatings. In contrast, the success of synthetic adhesives in wet environments requires carefully cleaned adherends, which often must also be chemically treated and/or partially dried.[3] Furthermore, preliminary immunological studies on marine adhesive proteins revealed that they are poor antigens and thus excellent candidates for in vivo medical applications.[4] An understanding of the materials and mechanisms used by mussels and barnacles to adhere to underwater surfaces would be valuable for the design and synthesis of superior moisture-resistant adhesives.

This premise has driven much of the research on marine adhesives. Decades of investigation into this field have led to the discovery of many marine organisms that secrete adhesive materials. These organisms include varieties of mussels,[5] barnacles,[6] and tube worms (polychaetes),[7] which have different environmental needs and subsequent uses for the adhesives they produce. However, they are alike in that the materials they use for adhesion and cementing contain many of the same building blocks and apparently operate by similar mechanisms. Adhesive precursor proteins have been isolated and sequenced from most of these organisms; a partial list is given in Figure 1. It is important to note that these consensus repeats are just that and that considerable variation is present in the sequence of each protein. The repetitive polypeptides have basic isoelectric points (due to lysine residues), flexible conformations (due to high percentages of small glycine and serine residues), and high levels of the amino acid 3,4-dihydroxyphenyl-L-alanine, DOPA (Figure 2).[5] The DOPA residues are believed to be primarily responsible for (i) chemisorption of the polymers to surfaces underwater and (ii) covalent cross-linking of the adhesive.[8]

Natural adhesive precursor protein has been extracted from the blue mussel, *Mytilus edilus*, and when this material was spread on culture plates and oxidized with mushroom tyrosinase, it formed an adhesive that could be used for cell attachment and growth.[9] It has also been shown to be effective as an adhesive for the bonding of wet tissue samples.[10] The adhesive protein was found to be nontoxic; however, the toxicity of the enzymatic oxidizing agent was problematic. Alternatively, synthetic DOPA-containing polypeptides have been reported by Yamamoto and co-workers. They synthesized DOPA homopolymer as well as sequence-specific copolymers of DOPA with L-lysine and L-glutamic acid.[11] They have also reported random L-lysine/L-tyrosine copolymers[12] and the synthesis of random copolypeptides that contain as many as 18 different amino acids, including DOPA.[13] Studies on the cross-linking and adhesive properties of these polymers were limited. Initial adhesive studies were performed using iron and Al_2O_3 adherends with no oxidizing agent.[14] More detailed studies focused on L-lysine/L-tyrosine random copolymers and complex random copolymers where tyrosinase enzyme was used as the oxidizing agent.[12] The adhesive systems were studied in water and diluted synthetic seawater and were found to form adhesive bonds, although conversion of tyrosine residues to DOPA was inefficient. Successful oxidation was achieved only when the polymer chains were cleaved with chymotrypsin.

Our efforts in this area are focused on the design and synthesis of simplified adhesive polymers that incorporate only the essential functional components of the marine proteins. We especially wanted to test the premise that functionality, and not amino acid sequence, was the only feature necessary for moisture-resistant adhesion. Using the known compositions of the natural

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Organism	M_n	Consensus Repeat
Phragmatopoma californicia	35,000	ValGlyGlyDOPAGlyDOPAGlyAlaLys
Mytilus edulis	130,000	AlaLysProSerTyrdiHypHypThrDOPALys
Geukensia demissa	130,000	ThrGlyDOPAGlyProGlyDOPALys
Aulacomya ater	130,000	AlaGlyDOPAGlyGlyLeuLys
Dreissena polymorpha	76,000	GlyProDOPAValProAspGlyProTyrAspLys
Brachidontes exustus	105,000	GlyLysProSerProDOPAAspProGlyDOPALys

Figure 1. Sequences of some naturally occurring marine adhesive protein repeats.

Figure 2. Oxidation of L-DOPA to DOPA quinone.

adhesive proteins, we prepared sequentially random copolypeptides through copolymerization of a few select α-amino acid *N*-carboxyanhydrides (NCAs) (Scheme 1). NCAs are readily prepared from amino acids by phosgenation and can be polymerized into high molecular weight polypeptides via successive ring-opening addition reactions that liberate carbon dioxide.[15] NCA polymerizations allow the preparation of multigram quantities of polymer, and the monomers readily copolymerize, allowing copolymer composition to be easily varied. We also wanted to study the oxidation of these polymers in detail since this chemistry is a key feature of the DOPA functionality. Thus we oxidized our copolymers under a variety of conditions and evaluated the effect of oxidant on both cross-linking and adhesive capabilities. The role of oxidation in these adhesives has not received much attention in the past: the complexity of the full-length proteins and ambiguities associated with oxidation of tyrosine residues to DOPA and/or quinone has hindered characterization of the oxidized polymers. Our functionally simple copolymers allowed us to make strong correlations between oxidation and physical behavior.

Experimental Section

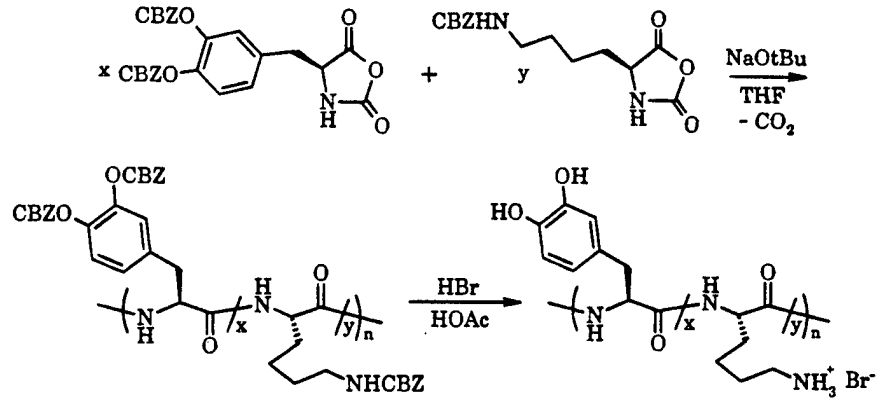
General Information. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on a Spectra Physics Isochrom liquid chromatograph pump equipped with a Wyatt DAWN DSP light-scattering detector and Wyatt Optilab DSP. Separations were effected by 10^5, 10^4, and 10^3 Å Phenomenex 5 μm columns using 0.10 M LiBr in DMF as eluent. NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. A Mettler TA3000 thermal analysis system was used for TGA measurements on the polymers. All IR samples were prepared on NaCl plates, and IR spectra were recorded on a Perkin-Elmer model 1615 FTIR. Optical rotations were measured using a JASCO P1020 digital polarimeter equipped with a sodium lamp source. Elemental analyses were performed by the Marine Science Institute Laboratory at the University of California, Santa Barbara. L-DOPA (99%), benzyl chloroformate (97%), N_ϵ-carbobenzyloxy-L-lysine (98%), sodium hydride, *tert*-butyl alcohol, and 33 wt % hydrobromic acid in glacial acetic acid (Technical grade) were obtained from Acros Organics. Mushroom tyrosinase was obtained from Sigma Chemical Co. Phosgene was purchased from Fluka. Phosphorus pentachloride (95%) was obtained from Aldrich Chemical Co. Hexanes and THF were distilled from sodium/benzophenone in an inert atmosphere and stored under nitrogen. Sodium *tert*-butoxide was prepared from the reaction of NaH and *tert*-butyl alcohol in THF. The product was dried under vacuum and stored in a glovebox.

Amino Acid *N*-Carboxyanhydrides and Polypeptides. N_ϵ-Carbobenzyloxy-L-lysine *N*-carboxyanhydride, **1**, was prepared by using phosgene following literature procedures.[16] *O,O'*-Dicarbobenzyloxy-L-DOPA *N*-carboxyanhydride, **2**, was prepared from *N,O,O'*-tricarbobenzyloxy-L-DOPA and phosphorus pentachloride.[17] Poly(N_ϵ-carbobenzyloxy-L-lysine), **3**, and Poly(L-lysine•HBr), **4**, were prepared according to literature procedures.[16]

Poly(N_ϵ-carbobenzyloxy-L-lysine$_9$-*O,O'*-dicarbobenzyloxy-L-DOPA$_1$), 5. To a mixture of **1** (1.3 g, 4.3 mmol) and **2** (0.23 g, 0.48 mmol) in THF (15 mL) in a Schlenk tube was added 0.1 N sodium *tert*-butoxide (0.95 mL, 0.096 mmol) with stirring. The mixture was stirred for 1 day at room temperature, then 2 days at 40 °C, and finally 4 h at 80 °C. The product was precipitated by addition of ether and then dried in vacuo at room temperature overnight to give a white polymer (1.13 g, 85%). GPC: M_n = 167 000, M_w/M_n = 1.48. ^{1}H NMR (TFA-*d*): δ 7.60−7.20 (br m, −(NHCH-((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$*H*$_5$)C(O))$_9$−(NHCH(CH$_2$C$_6$*H*$_3$-(OCOOCH$_2$C$_6$*H*$_5$)$_2$)−C(O))$_1$−, 5.8H), 5.28 (br s, −(NHCH-((CH$_2$)$_3$CH$_2$NHCOOC*H*$_2$C$_6$H$_5$)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_3$-(OCOOC*H*$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 2.2H), 4.68 (br s, −(NHC*H*-((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_9$−(NHC*H*(CH$_2$C$_6$H$_3$-(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 1H), 3.30 (br s, −(NHCH-((CH$_2$)$_3$C*H*$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_9$−(NHCH(C*H*$_2$C$_6$H$_3$-(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 2H), 2.06−1.38 (br d, −(NHCH-((C*H*$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_3$-(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 5.4H). FTIR(THF): 1772 cm^{-1} (ν_{CO}, vs), 1723 cm^{-1} (ν_{CO}, vs), 1654 cm^{-1} (amide I, s br), 1544 cm^{-1} (amide II, s br). Anal. Calcd for C$_{13.5}$O$_3$N$_{1.9}$H$_{17.1}$: C, 64.58; H, 6.57; N, 9.48. Found: C, 63.94; H, 6.88; N, 9.55. $[\alpha]_D^{20}$ (DMF, c = 0.005) = +7.7.

Poly(L-lysine$_9$•HBr-L-DOPA$_1$), 6. To a solution of **5** (1.1 g, 4.0 mmol) in TFA (~10 mL) was added 4 equiv of 33% HBr in acetic acid (w/w) with stirring. The mixture was stirred for 1 h. The product was precipitated by addition of ether and then dried under a flow of nitrogen. The crude polymer was dissolved in a small amount of water/methanol (1:4), precipitated out by addition of ether, and then dried in vacuo at room temperature overnight. The polymer was dissolved in water and isolated by freeze-drying as a white fluffy solid (0.72 g, 88%). ^{1}H NMR (D$_2$O): δ 7.58 (br s, −(NHCH((CH$_2$)$_3$CH$_2$-NH$_2$•HBr)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_2$*H*(OH)$_2$)C(O))$_1$−, 0.1H), 6.90−6.70 (br m, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_9$−(NHCH-(CH$_2$C$_6$*H*$_2$H(OH)$_2$)C(O))$_1$−, 0.2H), 4.40 (br s, −(NHC*H*((CH$_2$)$_3$-CH$_2$NH$_2$•HBr)C(O))$_9$−(NHC*H*(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 1H), 3.10(br s, −(NHCH((CH$_2$)$_3$C*H*$_2$NH$_2$•HBr)C(O))$_9$−(NHCH-(C*H*$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 2H), 2.18−1.40 (br d, −(NHCH-((C*H*$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C-(O))$_1$−, 5.4H). Anal. Calcd for C$_{6.3}$O$_{1.2}$N$_{1.9}$H$_{12.6}$Br$_{0.9}$: C,

Scheme 1. Synthesis of Adhesive Copolypeptides Using NCA Monomers



36.72; H, 6.16; N, 12.91. Found: C, 35.18; H, 5.69; N, 11.43. The composition of the polymer was estimated to be 11 mol % DOPA by measurement of the UV/vis absorption of a polymer solution at 280 nm and comparison of the data to a standard absorption curve of **8**. $[\alpha]_D^{20}$ (H_2O, c = 0.005) = −61.7.

Recarbobenzyloxylation of 6. **6** (M_n = 182 000, 50 mg, 0.24 mmol) was dissolved in 1 mL of water in a 25 mL Schlenk flask under N_2. The polymer solution was stirred in an ice bath. (4 N, NaOH 0.34 mL) and benzyl chloroformate (77 μL, 0.55 mmol) were added to the polymer solution in three portions over a 30 min period. A white precipitate formed after addition of the first portion of benzyl chloroformate. After the final addition, the mixture was stirred for an additional hour. The polymer was isolated by filtration, washed with water, methanol, and ether, and then dried in vacuo at room temperature overnight (46 mg, 68%). The ^{1}H NMR spectrum of the product was identical to an unmodified sample of **5**. The molecular weight of the reprotected polymer was determined by GPC: M_n = 252 000, M_w/M_n = 1.54. Original M_n of protected polymer = 330 000.

Poly(N_ϵ-carbobenzyloxy-L-lysine$_4$-*O*, *O*′-dicarbobenzyloxy-L-DOPA$_1$), 7. To a mixture of **1** (1.1 g, 3.7 mmol) and **2** (0.46 g, 0.93 mmol) in THF (15 mL) in a Schlenk tube was added 0.1 N sodium *tert*-butoxide in THF (0.11 mL, 0.019 mmol) with stirring. The mixture was stirred for 1 day at room temperature, then 2 days at 40 °C, and finally 4 h at 80 °C. The product was precipitated by addition of ether and then dried in vacuo at room temperature overnight to give a white polymer (1.22 g, 87%). GPC: M_n = 186 000, M_w/M_n = 1.35. ^{1}H NMR (TFA-*d*): δ 7.58–7.30 (br m, −(NHCH((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 6.6H), 5.26 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 2.4H), 4.70 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 1H), 3.28 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 2H), 2.10–1.36 (br d, −(NHCH((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 4.8H). FTIR (THF): 1772 cm^{-1} (ν_{CO}, vs), 1722 cm^{-1} (ν_{CO}, vs), 1652 cm^{-1} (amide I, s br), 1543 cm^{-1} (amide II, s br). Anal. Calcd for $C_{16.2}O_{3.8}N_{1.8}H_{18.6}$: C, 65.00; H, 6.26; N, 9.62. Found: C, 64.31; H, 6.46; N, 8.32. $[\alpha]_D^{20}$ (DMF, c = 0.005) = +13.2.

Poly(L-lysine$_4$•HBr-L-DOPA$_1$), 8. To a solution of **7** (1.1 g, 3.7 mmol) in TFA (~10 mL) was added 4 equiv of 33% HBr in acetic acid (w/w) with stirring. The mixture was stirred for 1 h. The product was precipitated by addition of ether and then dried. The crude polymer was dissolved in a small amount of water/methanol (1:4), precipitated out by addition of ether, and dried in vacuo at room temperature overnight. The polymer was dissolved in water and isolated by freeze-drying as a white fluffy solid (0.74 g, 3.6 mmol 97%). ^{1}H NMR (D_2O): δ 7.52 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 0.2H), 6.96–6.64 (br m, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 0.4H), 4.36 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 1H), 3.05 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 2H), 2.05–1.30 (br d, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 4.8H). Anal. Calcd for 19% DOPA copolymer, 85% TFA salt, 15% HBr salt: C, 42.86; H, 5.52; N, 11.32. Found: C, 42.86; H, 5.92; N, 10.08. The composition of the polymer was estimated by ^{1}H NMR: DOPA content found = 19 mol % [mol % DOPA = 100 − mol % lysine. Mol % lysine = {100(integration of peaks at 2.05–1.30 ppm)/3}/(integration of peak at 3.05 ppm)]. $[\alpha]_D^{20}$ (H_2O, c = 0.005) = −39.7.

Thermal Analysis. The polymers were heated at a rate of 15 °C/min from 50 to 500 °C under N_2. The decomposition temperature (T_d) was determined as the temperature where each sample showed 5% weight loss.

	polymer					
	3	**4**	**5**	**6**	**7**	**8**
T_d(K)	247	306	206	288	206	290

Rheology. The rheological behavior of solutions of **8** under different oxidation conditions was studied using a rheometrics ARES Rheometer at room temperature. A cone and plate geometry was used in a dynamic time sweep experiment at a frequency of 2.0 rad/s and at 100% strain. The concentration of each polymer solution was 2.5 mg/mL. Solutions were prepared by mixing a 5.0 mg/mL solution of the copolymer in deionized water with an equal volume of oxidant solution. The viscosity of each adhesive is reported at the beginning of curing and at the onset of gelation. The gel point was determined by the time at which G' and G'' became parallel as functions of frequency.[18]

Tensile Shear Measurements. Adherend Preparation. Aluminum adherends (5052-H32) were treated with a mixture of water, H_2SO_4 (concentrated), and $Na_2Cr_2O_7$ (40:20:4) at 65–70 °C for 20 min. They were then rinsed with deionized water and air-dried. Steel adherends (A366) were polished with sandpaper (220 grit) and then rinsed with hexane followed by acetone. Poly(methyl methacrylate), polystyrene, and polyethylene adherends were sonicated in aqueous Alconox detergent (2% w/v) for 30 min and rinsed with deionized water. Glass adherends were prepared by attaching glass slides to stainless steel test pieces using epoxy resin, and the exposed glass surfaces were cleaned by soaking in an 2-propanol/KOH bath for 30 min followed by rinsing with deionized water (18 MΩ resistivity).

Test Sample Preparation. The adhesive polymer solution (40 mg/100 μL of solvent, unless otherwise noted) was spread on both adherend slides, which were then overlapped together with two Cu wires (0.06 mm diameter) placed as spacers between the adhesive joint. The overlapped samples were clamped together for 3 h to prevent motion and kept in a temperature-controlled oven for the specified time period. No attempt was made to maintain hydration of the samples.

Table 1. Synthetic Adhesive Copolymers and Control Polymers[a]

Composition	yield (%)	M_n	M_w/M_n	H_2O soluble
poly(CBZ-lysine), **3**	94	147 000	1.42	−
poly(lysine•HBr), **4**	97	103 000	1.45	+
poly(CBZ-lysine$_9$−DiCBZ-DOPA$_1$), **5**	85	167 000	1.48	−
poly(lysine•HBr$_9$-DOPA$_1$), **6**	88	120 000	1.52	+
poly(CBZ-lysine$_4$−DiCBZ-DOPA$_1$), **7**	87	186 000	1.35	−
poly(lysine•HBr$_4$-DOPA$_1$), **8**	97	113 000	1.39	+

[a] Molecular weights of protected polymers were determined by GPC in 0.1 M LiBr in DMF at 60 °C.

Tensile Shear Strength Measurement. The tensile strength was measured at room temperature with an Instron 1123 mechanical testing apparatus according to the ASTM D1002 method.[19] An Instron 5000 lb reversible load cell was used for the measurements. *Data acquisition & control version 3.00* (1994 University of California, Santa Barbara) was used to monitor the data output. The adherend size was 4 in. × 1 in. the loading rate was 0.05 in./min, the bond line thickness was 0.06 mm, and the area of the bond was 0.39 in.^{2}. Three samples were measured for each experiment, and the average of these values is reported. The ranges of the data points are plotted as error bars on the graphs.

Results

We have prepared simple copolypeptides of L-lysine and DOPA containing different compositions of the two monomers. We wanted to incorporate DOPA directly into the polymer to avoid complications associated with the oxidation of precursor tyrosine residues. L-Lysine was the other major component of our copolymers since it (i) is present in large quantities in marine adhesive proteins, (ii) is thought to be involved in protein cross-linking reactions,[20] and (iii) should provide good water solubility to the copolymers when the side chain amine is ionized. We prepared homopolymers of L-lysine and DOPA as well as a series of copolymers of varying compositions and molecular weights. Data for the side-chain protected and deprotected polymers are given in Table 1.

Di-carbobenzyloxy-DOPA NCA (di-CBZ-DOPA NCA) was found to only form short chains in low yield when polymerized using sodium *tert*-butoxide initiator. As carbobenzyloxy-L-lysine NCA (CBZ-L-lysine NCA) polymerized very efficiently, this suggested that the steric crowding around the DOPA monomer resulted in poor homopolymerization. DOPA, however, was found to incorporate well in copolymerization reactions: yields were near quantitative, and no short homopoly(DOPA) oligomers were formed. ^{1}H NMR and UV/vis quantification of the DOPA catechol functional groups confirmed that the bulk copolymer compositions were essentially equal to the comonomer feed compositions. Deprotection of the copolypeptides was accomplished using HBr in acetic acid. Reprotection of a deprotected lysine/DOPA copolymer with excess benzyl chloroformate followed by GPC analysis showed that there was only limited peptide chain cleavage in the deprotection step: molecular weight of the polymer after reprotection with benzyl chloroformate was ca. 75% of the original value. The white copolymers were all found to be soluble in aqueous buffers over a wide pH range (ca. 2−12). The DOPA-containing copolymers were stable in air for days under acidic and neutral conditions, but discolored rapidly in basic environments. Thermal analysis of the polymers showed negligible weight loss under N_2 up to ca. 200 °C. The polypeptides could be stored in a −40 °C freezer indefinitely with no discoloration or change in properties.

The first goal of this project was to verify that these simple copolypeptides would form cross-linked networks in aqueous environments, analogous to the natural adhesive proteins. Specifically, we wanted to evaluate the ability of copolymer solutions to form networks as functions of solution pH and oxidizing agent. The oxidizing agents utilized were air (O_2), $NaIO_4$, H_2O_2, and mushroom tyrosinase. The oxidizing agents were found to darken solutions of the DOPA-containing copolymers, resulting in formation of cross-linked networks. By variation of the oxidizing agent and pH, we were able to obtain systems where gelation times could be adjusted from seconds to days. The kinetics of gel formation and gel strength were followed by viscosity measurements (Table 2). Periodate, hydrogen peroxide, and base (pH = 12) formed gels fastest, with tyrosinase and aerobic oxidation being much slower. Hydrogen peroxide and base also gave the highest cross-link densities, as estimated by solution viscosities. From these experiments, it appeared that either basic aqueous solution or H_2O_2 would be the most efficient oxidants for our synthetic adhesive polymers.

Lap shear tensile adhesive measurements were also performed on the L-lysine/DOPA binary copolymers. The copolymers were tested under a variety of oxidizing conditions and were found to form moisture-resistant adhesive bonds to aluminum under all conditions (Table 3). If allowed to cure for a sufficient period of time (1 day), all oxidizing conditions gave adhesive bonds of nearly equivalent strength, which was proportional to the amount of DOPA in the copolymer. Copolymer containing 20% DOPA formed adhesive bonds nearly 10 times stronger than those formed with the control, pure poly-L-lysine. When the adhesives were allowed to cure for shorter periods of time, the choice of oxidant became significant. A comparison between aerobic and peroxide oxidation is given in Figure 3. As the peroxide concentration was increased, oxidation of the polymer was accelerated, resulting in strong bonds after only 3 h of curing. With aerobic oxidation, only very weak bonds were formed in this time period.

We also evaluated the ability of our copolymers to adhere to substrates besides aluminum. The survey of adherends included steel (A366), plastic (PMMA, PS, and PE), and borosilicate glass (Figure 4). The plastic adherends formed the weakest adhesive bonds of all adherends studied. It is likely the relatively nonpolar surfaces displayed by the plastics provided a poor substrate for chemisorption of the polar functionalities of the polymer. The polar surface of glass provided a better substrate, nearly equivalent to the metals. Steel adherends gave adhesive bond strengths that were comparable to those formed on aluminum (Table 3). The major difference from aluminum was that adventitious iron oxide present on the steel surface was able to act as an efficient polymer oxidant. Consequently, no additional oxidant was necessary to obtain fast oxidation on steel adherends. In particular, it was found to be unnecessary to scrupulously clean the steel surface since a thin coating of iron oxide promoted rapid curing of the adhesive. The rate of adhesive bond formation on steel can be seen in Figure 5, where reasonable bonds were obtained after ca. 6 h. Bond strengths on steel were also strongly influenced by the copolymer composition, with the 20% DOPA copolymer forming bonds an

Table 2. Viscosity Measurements[a]

oxidation conditions	tyrosinase[b]		pH = 12[c]		$NaIO_4$[d]		H_2O_2[e]		air (O_2)	
time (min)	0	48	0	4	0	3	0	6	0	180
η^* (Pa s)	0.004	0.047	0.118	0.196	0.082	0.085	0.005	0.144	0.008	0.198

[a] The copolymer used was **8**, M_n = 126 000. [b] 300 units of enzyme were added to a pH = 7 phosphate buffer solution (0.01 N phosphate). [c] 0.01 N phosphate buffer. [d] 0.04 N solution. [e] 1.5 wt % solution. The two time points represent the beginning of the experiment and the onset of gelation.

Table 3. Adhesive Strengths (MPa) of Polymers as a Function of Composition under Different Oxidizing Conditions at 35 °C for 1 Day[a]

oxidation conditions	aluminum					
	air	H_2O_2[b]	pH = 7[c]	pH = 12[c]	tyrosinase/pH = 7[d]	steel air
4 [144k]	0.58 (0.25)	0.61 (0.23)	0.49 (0.03)	0.65 (0.15)	0.56 (0.18)	0.44 (0.16)
6 [242k]	2.52 (0.78)	2.39 (0.49)	2.24 (0.38)	3.45 (0.48)	2.81 (0.73)	3.36 (0.63)
8 [255k]	4.32 (0.55)	4.29 (0.81)	4.02 (0.73)	3.75 (0.39)	4.70 (0.87)	4.00 (0.60)

[a] The ranges of the data points are given in parentheses. Polymer concentration = 40 mg/100 μL. The polymer molecular weight is given in brackets. [b] The amount of H_2O_2 (0.3 wt %) used was 0.5 mL/g polymer. [c] Final concentration of phosphate buffer was 0.025 M. [d] The concentration of enzyme was 5 units/mg polymer.

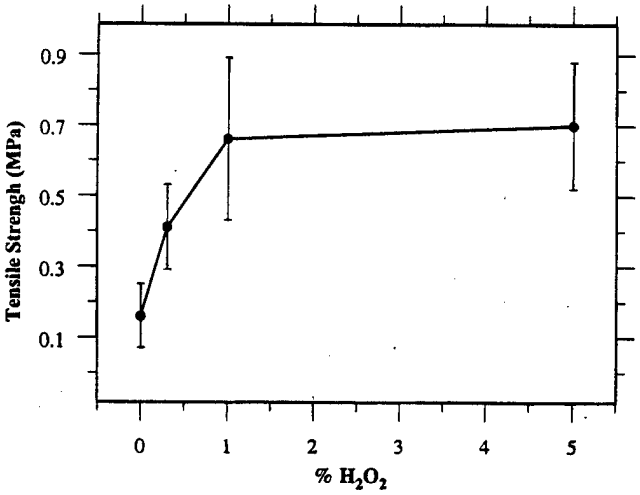


Figure 3. Adhesive strength as a function of H_2O_2 concentration for **8** (M_n = 98 000) on aluminum at 35 °C after 3 h cure time. The polymer concentration was 400 mg/mL of H_2O solution.

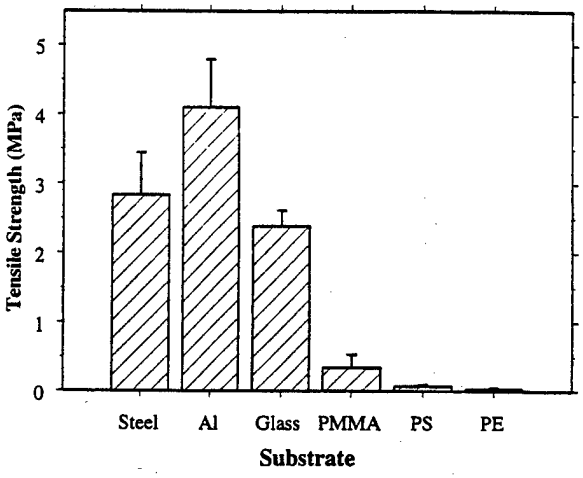


Figure 4. Adhesive strength of **8** (M_n = 98 000) on various substrates at 40 °C after 1 day cure time. The polymer concentration was 400 mg/mL of H_2O solution. The samples were cured in air. PS = polystyrene. PE = polyethylene. PMMA = poly(methyl methacrylate).

order of magnitude stronger than those formed with poly(L-lysine). These results correlate well with the measurements on aluminum in showing the importance of DOPA in adhesive formation.

In addition to copolymer composition and oxidant, we also studied the effects of cure temperature, copolymer molecular weight, and copolymer concentration on adhesive strength. We found that increasing the solution concentration of copolymer in adhesive formulations resulted in increased bond strengths on steel adherends (Figure 6). Presumably, this result was simply due to the increase in quantity of polymer within the adhesive joint. When the adhesives were cured at different temperatures, a large effect on ultimate bond strength was observed (Figure 7). Highest strengths were observed when the adhesives were cured at temperatures greater than 40 °C; shear strengths being more than double the values obtained at 20 °C. Oxidation occurred at all temperatures; however, curing of the adhesive may be more extensive at elevated temperatures. Finally, we found that adhesive bond strengths were also highly dependent on the molecular weight of the copolymer. Tensile strengths increased almost linearly with copolypeptide molecular weight (Figure 8), likely due to increased chain entanglements and cross-links.

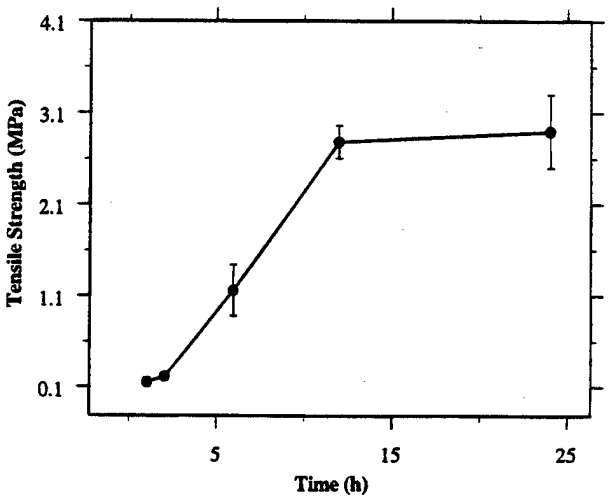


Figure 5. Adhesive strength as a function of cure time for **8** (M_n = 186 000) on steel at 40 °C. The polymer concentration was 400 mg/mL of H_2O solution.

Summary

We have reported the synthesis of copolymers of DOPA and L-lysine that display moisture-resistant

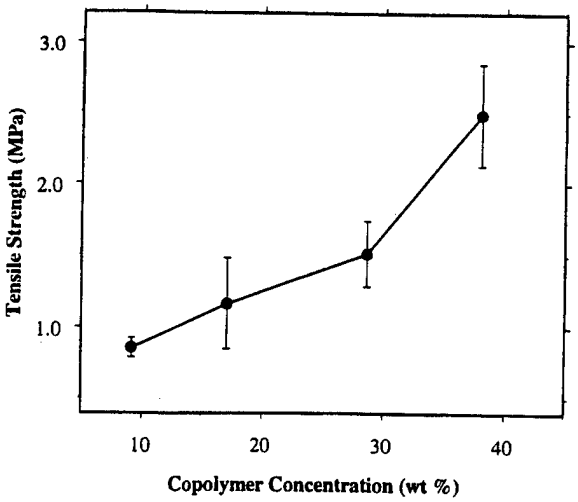


Figure 6. Adhesive strength as a function of copolymer concentration for **8** (M_n = 98 000) on steel at 40 °C after 1 day cure time.

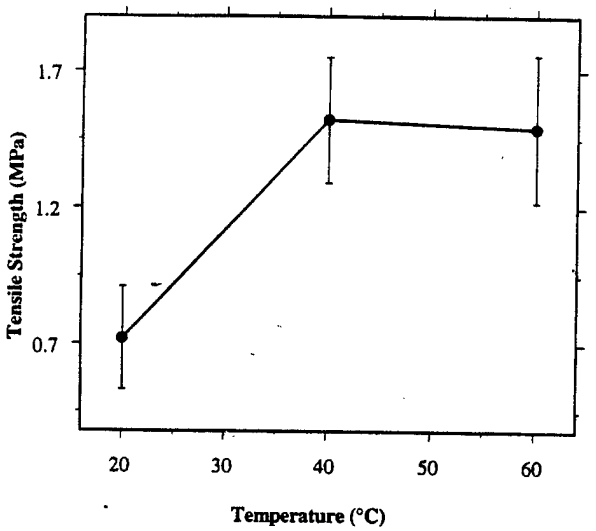


Figure 7. Adhesive strength as a function of cure temperature for **8** (M_n = 98 000) on steel after 1 day cure time. The polymer concentration was 400 mg/mL of H_2O solution.

adhesive properties when suitably oxidized. The adhesive bonds were all able to form when aqueous solutions of the polymers were applied to the substrates. Most of the water was found to evaporate from the bond during curing. However, since no effort was made to drive off all of the water, the adhesives likely retained some moisture of hydration. The choice of oxidant was partially substrate specific, since in some cases, as with steel adherends, no external oxidant is required. On other substrates, different oxidants can be chosen to satisfy particular requirements for curing time. Generally, hydrogen peroxide was found to be the most versatile oxidant when fast curing is required. Our system consisting of synthetic polypeptides and chemical oxidants thus provides an alternative to natural marine adhesive proteins and oxidizing enzymes for formation of adhesive bonds in water.[21] The advantages of our system include the incorporation of inexpensive oxidizing agents and the ready availability of large quantities of adhesive polymer of consistent quality. Furthermore, through adjustment of copolymer composition, molecular weight, or curing conditions, the adhesive properties of these synthetic copolypeptides can be readily tuned for specific applications.

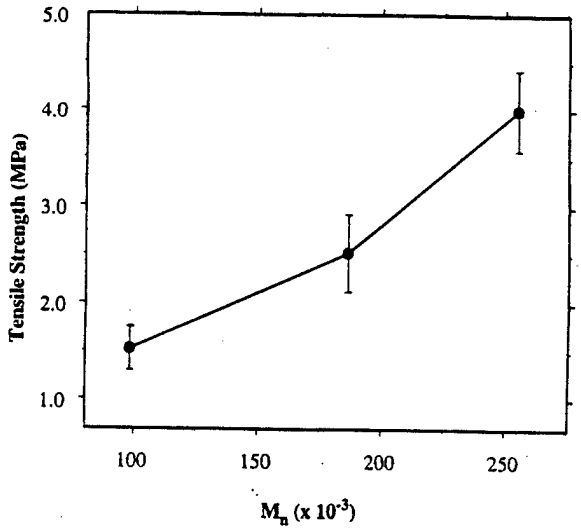


Figure 8. Adhesive strength as a function of copolymer molecular weight for **8** on steel at 40 °C after 1 day cure time. The polymer concentrations were 400 mg/mL of H_2O solution.

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Mussel byssus and biomolecular materials

Timothy J Deming

Mussel adhesive proteins are remarkable materials that display an extraordinary capability to adhere to substrates underwater. Recent investigations from groups with quite diverse areas of expertise have made substantial progress in the identification of the genes and proteins that are involved in adhesive formation. These discoveries have led to the development of recombinant proteins and synthetic polypeptides that are able to reproduce the properties of mussel adhesives for applications in medicine and biotechnology.

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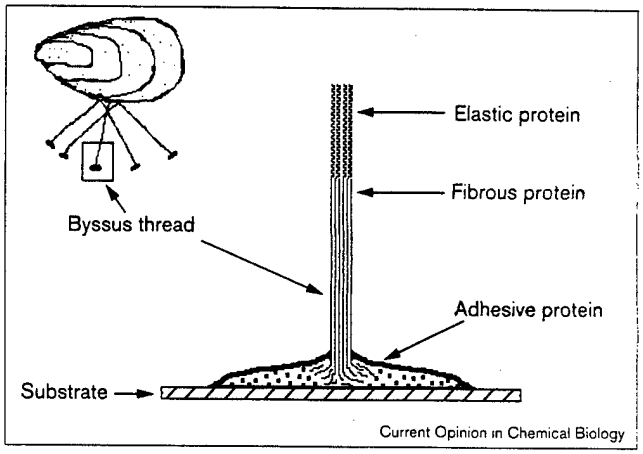
Abbreviation
DOPA 3,4-dihydroxyphenyl-L-alanine

Figure 1



A cartoon showing the structure and composition of byssus attachment threads for *Mytilus edulis*.

Introduction

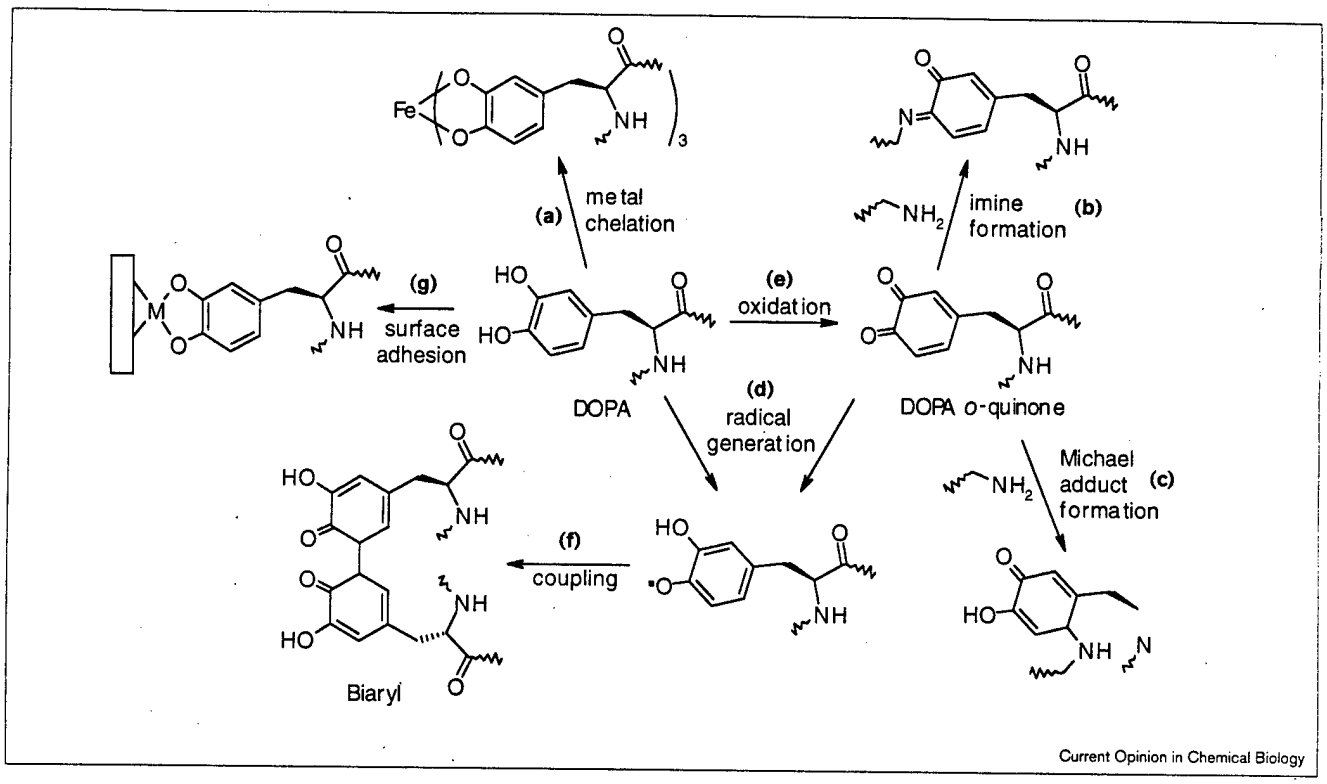
Permanently attached marine organisms are remarkable in that their ability to form moisture-resistant adhesive bonds is unrivalled by human efforts. These adhesives are unusual in that they can function over wide temperature ranges, fluctuating salinities, humidities, and in the tides, waves and currents of marine environments [1]. These glues are able to form permanent bonds in a few seconds to a wide variety of substrates with complex and often irregular surface coatings. In contrast, the success of synthetic adhesives in wet environments requires carefully cleaned adherends that often must also be chemically treated and/or partially dried [2]. In mussels, there are also the byssi, the threads that anchor the organism to a substrate, which display a unique gradient of mechanical properties, from stiff to elastic, along the length of each fiber (Figure 1) [3••]. These properties hold the organism fast, but provide enough flexibility to prevent brittle fracture of the fibers in the tidal environment. Mussels have evolved advanced structural materials that rival and in many ways surpass man-made materials. Certainly, knowledge and understanding of the materials and mechanisms used by mussels and barnacles to adhere to wet surfaces would be valuable for the design and synthesis of superior synthetic adhesives for wet environments, such as biological surfaces.

Decades of investigation into this field have led to the discovery of many marine species that secrete adhesive materials. These organisms include many varieties of mussels [4–7], barnacles [8], and tube worms (polychaetes) [9], which have different environmental needs and subsequent uses for the adhesives they produce, but are alike in that the materials they use for adhesion and cementing contain many of the same building blocks and apparently operate by similar mechanisms. Initial studies on mussels concentrated on secretion processes relevant to adhesive bond formation and on the location of the secretory organs and ducts that process and apply the adhesive mixtures [10]. Attempts were also made to characterize the adhesives themselves, yet the insolubility and intractability of the materials hampered these studies, which were limited to complete disintegration of the cured cements and compositional analysis of the fragments [1]. These analyses indicated that the byssus and adhesive plaques were composed primarily of collagen and an adhesive protein, and that some of the amino acid components of the adhesive protein had been post-translationally modified [11].

In subsequent work [1], researchers were able to isolate precursor proteins from secretory glands that had been identified through histochemical and ultrastructural studies. Isolation of these proteins was advantageous since they were soluble individual polymer chains and could (in some cases) be purified and analyzed for composition, sequence and structure. These proteins were isolated prior to processing steps, however, and as the processing of structural proteins is very important in the development of ultimate material properties, no unequivocal correlation could be made between these precursors and the active adhesive material.

The isolation and sequencing of adhesive precursor proteins from mussels is an ongoing and active area of research. As with most structural proteins, these precursors typically have repetitive sequences and have been found to range from very short to quite long chains (i.e. molecular weights of 5–120 kDa) [12••]. The one common feature of all adhesive

Figure 2



Hypothetical adhesion, oxidation and cross-linking reaction pathways for peptidyl DOPA and DOPA *ortho*-quinone residues. **(a)** Chelation of catechol to a metal. **(b)** Coupling of DOPA-quinone with a pendant amine group to form an imine crosslink. **(c)** Coupling of DOPA-quinone with a pendant amine group in a Michael addition reaction to form a cross-link. **(d)** Reaction of DOPA or DOPA-quinone to form free-radical-containing species. **(e)** Oxidation of DOPA to DOPA-quinone. **(f)** Coupling of DOPA-based radicals to form dimeric cross-linked products (biaryl). **(g)** Adsorption of DOPA to metal- or metal-oxide-bearing surfaces through hydrogen bonding or other means. M, metal.

proteins isolated from mussels is the presence of high levels of the amino acid 3,4-dihydroxyphenyl-L-alanine (DOPA) (Figure 2) [4–9]. Indeed, DOPA is found in all known adhesive proteins from four different phyla [13,14]. The DOPA residues are key components that are believed to be primarily responsible for chemisorption of the polymers to substrates underwater and covalent cross-linking of the adhesive [13,14].

Studies on adhesion mechanisms of marine adhesive proteins have unfortunately consisted mostly of analogy and hypothesis. The catechol sidechain of DOPA is believed to be able to displace tightly bound water molecules from substrates, since catechols are able to form strong hydrogen bonding interactions (Figure 2g) [15]. In addition, the strong chelating power of catechols for metal ions and metal oxides (which are present in the rock to which mussels attach) has been proposed to explain the extraordinary adhesive capability of the adhesive proteins (Figure 2a) [16]. These hypotheses were substantiated by analogies to the chemistry of unsubstituted catechol complexes [17]. The dearth of experimental data on protein adhesion stems from the complexity associated with characterizing complexes containing a full-length adhesive protein and even single repeats of the polymeric sequence [18]. The cross-linking capability of DOPA residues is believed to result from the action of a catechol oxidase enzyme, which converts tyrosine residues to DOPA and subsequently to *ortho*-quinones (Figure 2e) [19]. The quinone sidechains can subsequently undergo a variety of hypothetical reactions to form interchain cross-links (Figures 2b,c,d,f) [13,14].

There has been some research toward understanding the oxidation and cross-linking processes. Rzepecki and coworkers [20] have been able to identify α,β-dehydro-DOPA as a possible initial reaction product in the oxidation of DOPA in small peptides (Figure 2e) [20]. While this is a significant result, illustrating the importance of using short peptides for analysis of these adhesives, a cross-linked DOPA-containing final product was not identified. NMR studies were undertaken to evaluate the possibility of cross-linking between DOPA units and the ε-amino groups of lysine in biologically derived adhesive proteins through the use of ^{13}C- and ^{15}N-labeled lysine [21]. These experiments showed no observable cross-links resulting from the lysine ε-amino group, making one of the most widely published hypotheses on cross-link formation appear unlikely (Figure 2c). Michael addition cross-links between dopamine and histidine units have been observed in insect cuticles using ^{13}C- and

Table 1

Sequences of some mussel foot proteins.

Mussel foot protein	Major consensus repeats	Estimated mass
Mefp-1	AKPSYPPTYK	>100 kDa
Mefp-2	XXNXCXPNPCKNXGXCXXX GXXXYXCXCXXGYXGXXC	42–47 kDa
Mefp-3	ADYYGPNYGPPRRYGGGNYNRY NGYGGGRRYGGYKGWNNGW NRGRRGKYW	6 kDa
PreCol-P	XGGPG and G(P/X)(P/X)	95 kDa
PreCol-D	AAAXGGGX and G(P/X)(P/X)	80 kDa

Byssus plaque (Mefp) and thread (PreCol) proteins isolated from *Mytilus edulis*. **P**, *trans*-4-hydroxy-L-proline; P, *trans*-2,3-*cis*-3,4-dihydroxy-L-proline; R, 4-hydroxy-L-arginine; X, variable residue; Y, DOPA.

^{15}N-NMR techniques (AM Christensen *et al.*, see Note added in proof). It is uncertain, however, if these results have any connection with the cross-linking chemistry of marine adhesives. The abundance of potential reactions of DOPA and *o*-quinones creates considerable uncertainty as to the roles played by these species in the cross-linking process [13,14].

Overall, many research groups have made excellent investigations into the identification of natural marine adhesive proteins from different organisms. The results of these efforts have provided a basis for many hypotheses on the action of these materials, setting the stage for more detailed studies. Recent advances in protein isolation and localization, for both adhesive and fibrous byssus proteins, are reviewed here. Also reviewed are recent efforts made to produce biomimetic adhesive materials, using both biology and chemistry, where the materials have been modeled after mussel adhesive proteins. Such efforts may ultimately lead to efficient, cost-effective wet-environment adhesives.

Advances in adhesive protein characterization

In recent years, researchers, most notably from the Waite group, have made tremendous advances in identification of mussel adhesive proteins. The protein sequences were deduced primarily through examination of cDNA fragments obtained from isolated mRNA via reverse transcriptase/polymerase chain reaction techniques [22]. Using this strategy, a number of new adhesive protein sequences have been identified for many varieties of mussel. The limitation of this methodology is that the end applications and potentially modified states of the proteins are unknown: the adhesive nature of each protein was deduced by examination of DOPA content and sequence homology with other adhesive proteins [23].

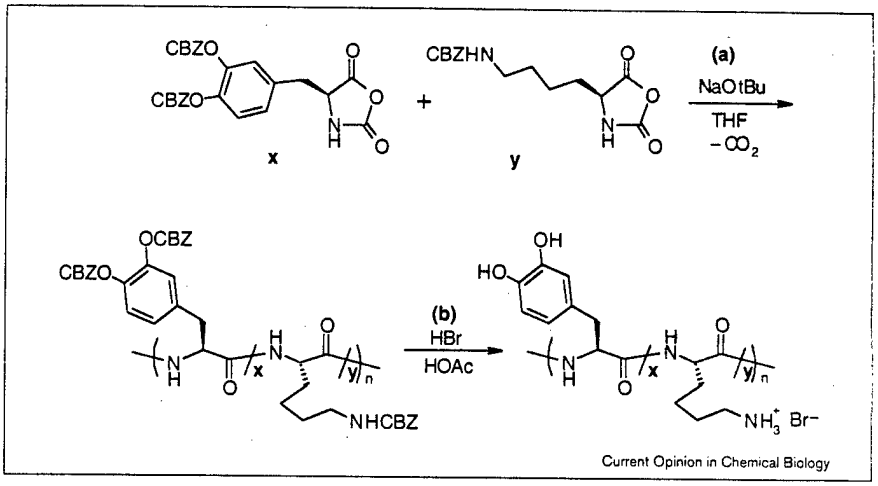
These proteins typically are homologous to the foot proteins of *Mytilus edulis*, which has been the most thoroughly studied mussel. Mefp-1 is a high molecular weight protein which contains up to 80 repeats of the decapeptide consensus sequence shown in Table 1 [24]. Analogs of Mefp-1 have been identified for *Mytilus galloprovincialis* [24] and *Mytilus coruscus* [25], both of which show a certain degree of homology to Mefp-1 in terms of maintaining the x-Y-x-x-x-Y-K motif (K = lysine; x = variable residue; Y = DOPA). It is important to note that these consensus sequences are just that, and that considerable sequence variability is present both between different organisms and within the sequence of any individual organism [13]. Mefp-2 is the most abundant foot protein and is constructed of a repetitive epidermal growth factor motif placed in between acidic DOPA-rich domains [26]. Mefp-3 is made up of a family of at least ten short proteins that are rich in both arginine residues and DOPA [27]. Certainly, the number and variety of mussel foot proteins makes predictions correlating sequences to functions a daunting task.

A better way to identify the proteins that are actively involved in adhesive processes would be to isolate them directly from the adhesive plaques. This direct approach has historically been prohibited by the cross-linking and near-complete insolublization of these proteins in the plaques. The problem has been circumvented by recent observations that the cross-linking of adhesive plaques is retarded at low temperatures (4–8°C) [28]. Extraction of mussel plaques produced at these temperatures has allowed the isolation and sequencing of new families of adhesive proteins. Using this strategy, Waite and Ross [29] have been able to identify Mefp-1 and Mefp-3 directly from the plaques. Characterization of these proteins has revealed some unique amino acid modifications not detectable from cDNA sequences, notably the presence of *trans*-2,3-*cis*-3,4-dihydroxyproline in Mefp-1 [30] and 4-hydroxyarginine in Mefp-3 [27]. It is unknown what roles these unusual amino acids play in cement formation; however, their highly polar nature hints that they might be critical for good adhesion.

More importantly, these proteins could be localized by their detection and isolation from various portions of the plaques. Thus, for the first time, the role of a specific protein could be assigned on the basis of its location in the plaque. Since Mefp-1 was found to coat the entire

Figure 3

Synthesis of adhesive co-polypeptides using α amino acid *N*-carboxyanhydride monomers. **(a)** Co-polymerization of lysine and DOPA anhydride monomers to yield the protected lysine–DOPA co-polymer. **(b)** Removal of protecting groups (CBZ) from the polymer to give the water soluble co-polypeptide. CBZ, carbobenzyloxy protecting group; HOAc, acetic acid; NaOtBu, sodium *tert*-butoxide; THF, tetrahydrofuran; x and y, relative amounts of DOPA and lysine monomers, respectively.



byssus thread, it has been proposed that this protein functions as a lacquer that forms a tough protective coating over the fiber within [31]. Using matrix-assisted laser desorption–ionization time-of-flight mass spectroscopy, Waite and Ross [29] were able to detect only Mefp-3 at the adhesive plaque–substrate interface. This result was taken to imply that the Mefp-3 family may act as the key proteins that prime various substrates for adhesion. While it might be premature to assume that the roles of these proteins have been conclusively identified, these results do provide some much needed new data to help sort out this system.

Advances in byssus characterization

The majority of early studies on marine adhesion focused almost entirely on the adhesives themselves, overlooking the connecting threads. The byssus threads are remarkable materials in their own right in that they perform extremely well in maintaining the structural integrity of the tether between organism and point of attachment [13]. The variety of forces these threads experience in the tidal environment require them to be both stiff and tough. As a result, the mussel has engineered fibers possessing a precise balance of strength and flexibility and which can be synthesized in less than 5 minutes.

Waite and co-workers have made considerable progress in the analysis of the fibrous component of mussel byssus. Because of its highly cross-linked structure, the only way to isolate fibrous proteins from byssus has been to partially hydrolyze the proteins by extensive treatment with pepsin, followed by acid extraction of the fragments [32]. Using this approach, Qin and Waite [33] were able to isolate two collagen-like proteins, Col-P and Col-D, which both exist as homotrimers [33]. The proteins show a complimentary distribution along the length of the thread, with Col-P dominating on the proximal end and Col-D dominating on the distal end. Presumably, this distribution of protein correlates directly with the physical properties of the threads, namely that they are elastic on the proximal end and rigid at the distal end.

These correlations were supported by isolation of cDNAs encoding Col-D and Col-P precursor proteins, PreCol-D and PreCol-P, respectively (Table 1). The sequence of PreCol-D revealed silk fibroin-like domains adjacent to the collagen-like domains [34•]. Presumably the silk-like domains stiffen the threads by formation of crystalline β sheet structures, as found in natural silk fibers. The sequence of PreCol-P revealed a collagen-like domain which was flanked by sequences resembling elastin (i.e. contain a high percentages of glycine, proline and bulky hydrophobic residues) [35••]. It has been proposed that these elastin-like domains are responsible for the flexibility of byssus threads at the proximal end. While these investigations of byssus fiber are still in their early stages, the breakthroughs obtained to date have already revealed new intriguing strategies by which nature assembles structural materials.

Applications of marine adhesives

In addition to the biochemical interests in marine adhesives, a substantial impetus behind understanding these adhesives is the potential to utilize these materials for technological applications. Numerous groups have investigated the use of mussel adhesive proteins for such applications as surgical tissue adhesives [36], dental cements [37] and binding agents for cell culturing [38]. While the proteins, most notably Mefp-1, have generally performed exceedingly well in tests for these applications, commercialization has been hampered by lack of an inexpensive source of large quantities of this protein.

To overcome limitations in isolation of the proteins from mussels, a few research groups have expressed recombinant proteins based upon Mefp-1 in microbial hosts. Using DNA templates that code for 20 copies of the decapeptide repeat of Mefp-1, researchers from Allied Signal (Morristown, New Jersey, USA) produced an adhesive protein precursor

in *Escherichia coli* [39]. Subsequent treatment of this precursor with mushroom tyrosinase *in vitro* converted encoded tyrosine residues to both DOPA and quinone groups, resulting in both cross-linking and adhesive formation. These methods were limited by not being able to introduce DOPA residues directly into the proteins, necessitating the use of oxidizing enzymes, and by tedious purification protocols which limited the yield of protein [39].

As a means to circumvent these problems, myself and coworkers [40] have explored the use of purely chemical methods to prepare adhesive protein analogs. These efforts focused on the design and synthesis of simplified adhesive polymers that attempted to incorporate only the essential functional components of the marine proteins. These polymers tested the premise that functionality, and not amino acid sequence, was the only feature necessary for moisture-resistant adhesion in these materials. Using the known compositions of the natural adhesive proteins, we prepared sequentially random co-polypeptides through co-polymerization of a few select ε-amino acid *N*-carboxyanhydrides (Figure 3) [41•]. These polymerizations allowed the preparation of multigram quantities of polymer, and, since the monomers readily co-polymerize, allowed copolymer composition to be easily varied.

Simple two-component co-polypeptides of L-lysine and DOPA with different compositions of the two monomers were prepared [41•]. Lap shear tensile adhesive measurements were then performed to measure the adhesive capabilities of these co-polymers on a variety of substrates. The co-polymers were found to adhere strongly to wet metal and glass surfaces. The co-polymers were also cured under a variety of oxidizing conditions, all of which gave adhesive bonds of nearly equivalent strength, which was proportional to the amount of DOPA in the co-polymer. Co-polymer containing 20% DOPA formed adhesive bonds nearly ten times stronger than those formed with the control, pure poly-L-lysine. These biomimetic systems were found to form strong moisture-resistant adhesive bonds without the need for enzymatic oxidation or other biological components [41•]. These results showed that incorporation of key elements of mussel adhesive proteins into synthetic polymers was a viable means to prepare effective adhesives for wet environments.

Conclusions

Currently, there is research underway in many areas relating to marine adhesives. These efforts range from isolation and identification of the components of mussel byssus to the development and engineering of new materials based on these precedents. Overall, much is being learned in this field, which may translate into new paradigms for biological structural materials, as well as into the design of man-made materials.

Note added in proof

The paper referred to as (AM Christensen *et al.*) was originally omitted during writing and can now be found as [42].

Acknowledgements

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Role of L-3,4-Dihydroxyphenylalanine in Mussel Adhesive Proteins

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Role of L-3,4-Dihydroxyphenylalanine in Mussel Adhesive Proteins

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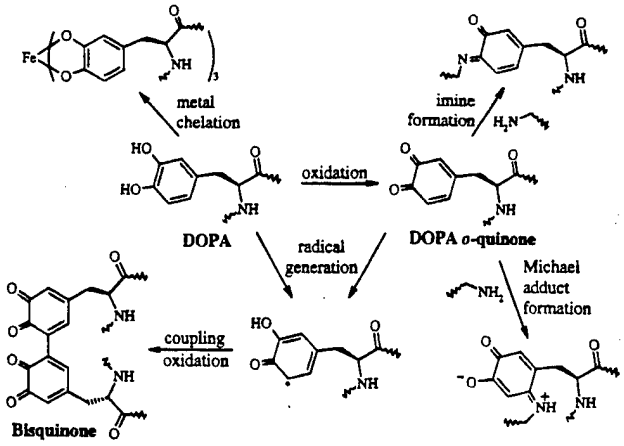
Mussel adhesive proteins (MAPs) have drawn interest for their ability to form strong adhesive bonds to a variety of substrates in wet environments.[1] There have been efforts to develop biomedical adhesives from these proteins, yet this has been hampered by the difficulty of isolation from biological sources.[2] Synthetic polymers are a potential source of functionally equivalent adhesives, yet little is known about how the MAPs function and therefore what must be incorporated into a synthetic analogue. The catechol functionality of L-3,4-dihydroxyphenylalanine, DOPA, residues is thought to be responsible for adhesion and cross-linking of the proteins; however, the mechanisms for these processes are unknown. The potential involvement of L-lysine and other polar residues in these reactions further complicates analytical efforts.[1] Speculation on the key components of these materials has been limited since no cross-link bond or specific bond to a substrate has been identified as yet. Through analysis of amino acid derivatives and simple copolypeptides under adhesive curing conditions, we have determined that DOPA is the only functional element required to reproduce the properties of MAPs. Furthermore, the primary roles of both catechol and *o*-quinone forms of DOPA can be assigned to adhesive bonding and cross-link formation, respectively.

The most detailed studies on MAPs have focused on the blue mussel, *Mytilus edulis*. This organism anchors itself to surfaces by means of plaques on the ends of fibrous threads. A considerable variety of adhesive proteins have been isolated from uncured plaques. These proteins range in mass from ca. 5 to 120 kDa, and all contain high levels of DOPA (ca. 5−20 mol %).[3] Variants also contain elevated levels of other polar amino acids such as hydroxylated prolines, lysine, and 4-hydroxyarginine. The variability of these proteins, in terms of their chain lengths, sequences, and compositions, has made it difficult to identify the important components responsible for adhesion.

It is known that catechol oxidase enzymes are present in MAP secretions that convert the catechol groups of DOPA into highly reactive *o*-quinone functionalities.[4] Numerous reactions have been proposed for cross-linking of the quinones (Scheme 1), yet none of these have been experimentally verified in MAPs.[1] The most often cited reaction is the Michael addition of side-chain amino groups of lysine residues to a DOPA−quinone residue.[5] Although all attempts to detect this product to date have been unsuccessful, the importance of the Michael addition in quinone chemistry has generated strong support for lysine cross-linking in MAPs.[6]

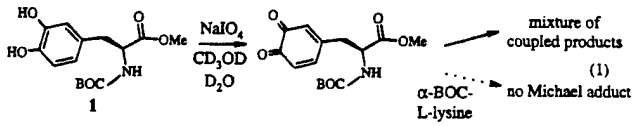
Our goal was to experimentally identify the roles of amino acids that are active in the adhesive chemistry of MAPs. Problems

Scheme 1. Hypothetical Cross-Linking Reaction Pathways for Peptidyl DOPA and DOPA *o*-Quinone Residues



associated with the complexity of polyfunctional proteins were avoided by studying small molecules and simple two-component copolypeptides. To connect the behavior of these model systems with that of MAPs, materials were analyzed using both molecular and macroscopic techniques (i.e., spectroscopy as well as measurement of bulk properties). Correlation of properties between small molecule and copolymer and then copolymer and protein thus connected the chemistry of individual amino acid components with the adhesive and cross-linking behavior of MAPs.

Initially, studies were focused on the oxidation and reactivity of single DOPA molecules. The methyl ester of *N*-BOC-DOPA, **1**, was reacted with $NaIO_4$ in pH 7.0 buffer, and the reaction was followed by ^1H NMR and UV/vis spectroscopy (eq 1). The



starting catechol was found to be quantitatively converted to the *o*-quinone within 5 min as expected (λ_{max} = 395 nm).[4] In the NMR reaction, a red DOPA-containing precipitate began to accumulate, as evidenced by a lack of aromatic protons in the NMR spectrum of the solution phase. In a dilute solution reaction followed by UV/vis spectroscopy, the quinone absorbance decreased in intensity over time and was gradually replaced by general absorption at higher wavelengths, also supportive of reaction of the quinone (Figure 1). It appeared that once the *o*-quinone groups were formed, they rapidly self-condensed to insoluble products.

When amines (e.g., α-*N*-BOC-L-lysine) were added to oxidized **1** in situ at pH 7.0, we observed no additional changes in the UV/vis spectra and no incorporation of amine into the product precipitates. Similar results were obtained when the reactions were performed under conditions similar to the tidal environment (pH 8.0). No evidence for Michael addition of primary amines to oxidized **1** was observed.[7] To determine the nature of the observed quinone self-condensation, we analyzed the precipitate formed in the oxidation of **1**. The product was found to be soluble in polar organic solvents (e.g., THF); however, ^1H NMR spectra in these solvents showed only numerous unassignable peaks. Size exclusion chromatography in THF showed that the products were low molecular weight compounds. These data were confirmed

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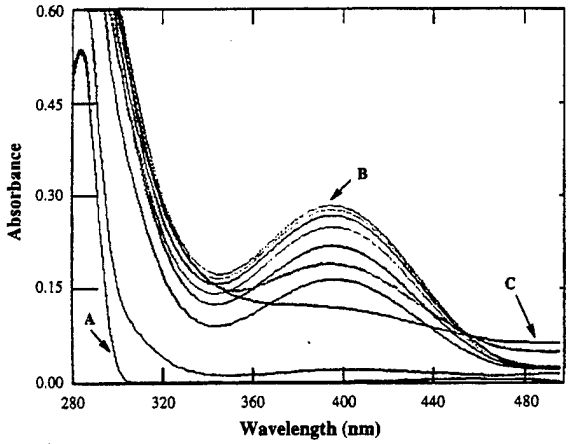

Figure 1. UV/vis spectrum of **1** mixed with $NaIO_4$ (10 equiv) in 0.025 M sodium phosphate at pH 7.0: (A) no oxidant; (B) 10 s after oxidant added; (C) 10 h after oxidant added.

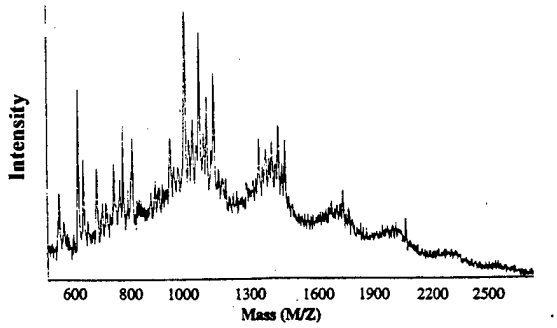

Figure 2. MALDI-TOF MS analysis of the oxidation products of **1**.

by MALDI-TOF mass spectra of the material. A series of DOPA oligomers were observed with masses ranging from 620 to 2620 Da, which correspond to dimers through octamers of the starting material (Figure 2). Thus, **1** couples to form bisquinone units (Scheme 1), which then condense with additional DOPA molecules to form higher oligomers.

To connect the chemistry of **1** with polymeric materials containing many DOPA residues, we studied simple copolymers of DOPA containing either L-lysine or L-glutamic acid. Statistical copolypeptides containing 5 mol % DOPA and the remainder either lysine or glutamic acid were prepared by standard procedures.[8] The resulting copolymers, poly(DOPA)$_{0.05}$(Lys·HBr)$_{0.95}$ (M_n = 148 000) and poly(DOPA)$_{0.05}$(Glu$^-$Na$^+$)$_{0.95}$ (M_n = 131 000), were both water soluble. DOPA reactivity in the copolymers was readily followed using UV/vis spectroscopy. Oxidation of each polymer with $NaIO_4$ in aqueous solution (pH 7) produced spectra that were intrinsically similar to one another, and to the data obtained for the DOPA model system. A quinone absorbance at 405 nm appeared initially, but over time decreased in intensity and was replaced by general absorption. With both copolymers, oxidation also resulted in gelation of the solutions. Viscosity measurements under oxidizing conditions showed that the copolymers formed networks of similar ultimate strength. This result indicated that the cross-link density, and thus the nature of the cross-links, was virtually identical for both copolypeptides. These data show conclusively that quinone curing in these copolypeptides does not require, and was not altered by, the presence of lysine residues.

We have previously shown that statistical copolypeptides of lysine and DOPA are able to form adhesive bonds in water comparable in strength to MAPs.[8] Bulk adhesive measurements on aluminum, where the bond was formed by oxidative curing of the polymer in water, were now undertaken for the DOPA–glutamate copolymer. It was found that this copolypeptide also formed moisture-resistant adhesive bonds (tensile strength 5.4(1.0) and 5.1(1.1) MPa for the lysine- and glutamate-containing copolymers at pH 7.0, respectively). As controls, poly(Lys·HBr) and poly(Glu$^-$Na$^+$) formed bonds of only negligible strength under similar curing conditions (0.6(0.2) and 0.6(0.4) MPa, respectively). Thus, it was evident that DOPA was the only residue required for both adhesion and cross-linking in these materials, which exhibit the key performance characteristics of MAPs.

To clarify the role of DOPA in the adhesion process, the bonding capability of a DOPA–lysine copolymer was measured in both the absence and presence of oxidant (H_2O_2). This experiment allowed separate evaluation of the abilities of DOPA–catechol and DOPA–quinone to adhere to wet surfaces. Bulk adhesive measurements of poly(DOPA)$_{0.05}$(Lys·HBr)$_{0.95}$ on aluminum under nitrogen with and without peroxide gave tensile strengths of 4.9(0.8) and 5.7(1.1) MPa, respectively. The bond strengths were similar, showing that the unoxidized catechol form of DOPA is primarily responsible for adhesion: addition of *o*-quinone groups did not improve, but rather weakened, bonding. By increasing the concentration of oxidant, the amount of catechol present for adhesion could be diminished. It was observed that rapid oxidation of copolymer solutions decreased adhesive-forming ability considerably (tensile strength 5.4(1.0) and 1.8(0.7) MPa for 0.5 and 5.0% peroxide, respectively). Furthermore, rapidly oxidized adhesives formed bonds that failed at the interface, as opposed to cohesive failure for slowly oxidized samples, illustrating that catechol is the active form of DOPA in surface adhesion.

In summary, our experiments have shown that the adhesion and cross-linking chemistry of MAP model copolymers is due primarily to the DOPA residues in the materials. These studies also revealed that the catechol functionality is primarily responsible for moisture-resistant adhesion and that the oxidized *o*-quinone functionality is primarily responsible for cross-linking. These insights can be used to optimize the properties of DOPA-containing polymers to create highly effective moisture-resistant adhesives for both industrial and biomedical applications.

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Supporting Information Available: Details of all experiments, synthesis of materials, NMR and GPC of oxidation products of **1**, and UV/vis spectra and viscometry of oxidized polymers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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A coaxial cable for the nanoworld

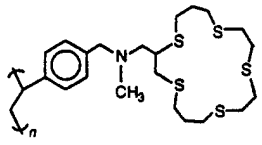
If the carbon nanotube is the vanilla ice cream of the nanotube world, then a new, more complex nanostructure unveiled by a Japanese-French team is the equivalent of spumoni. The researchers call their discovery a coaxial nanocable. It is a nanoscale version of the coaxial cable conventionally used to transmit electrical signals. The nanocable is a few tens of nanometers in diameter and up to 50 μm long. It consists of a crystalline core of silicon carbide (β-SiC) covered with an amorphous silicon oxide (SiO_2) layer, which is sheathed in distinct graphitic shells of boron nitride (BN) and carbon. This arrangement of concentric layers produces a semiconductor-insulator-metal or semiconductor-insulator-semiconductor sandwich that would be useful for nanoscale electronic devices, according to researchers Yuegang Zhang and Sumio Iijima of NEC Corp.'s Fundamental Research Laboratories in Tsukuba, Japan, and coworkers [*Science,* **281,** 973 (1998)]. They prepared the nanocable by reactive laser ablation of a powdered mixture of carbon, BN, SiO, and Li_3N heated to 1,200 °C. The electronic properties of the nanocable can be tailored by changing the starting materials, the researchers note.◀

On the road to pump-free dye lasers

A Japanese team has made progress in developing a dye laser that doesn't require external pumping—that is, excitation of the dye molecules by an external laser. In the 1970s, researchers tried to coax laser light out of dye molecules using electrochemiluminescence (ECL). In this process, electrons transfer between electrochemically generated anion and cation radicals, creating excited-state molecules that emit light before returning to the ground state. However, the rate of pumping was two orders of magnitude lower than required for laser action. Chemists Tsutomu Horiuchi and Osamu Niwa and physicist Noriyuki Hatakenaka at Nippon Telegraph & Telephone Corp. in Atsugi, Japan, now have created an ECL device that appears to generate laser light from dye molecules [*Nature,* **394,** 659 (1998)]. In their device, a dye solution flows between two electrodes only a few micrometers apart. The tiny gap enhances ECL intensity by greatly increasing the cycling of the redox reaction between the dye molecules, the authors say. Additionally, the device's microcavity apparently reduces the current threshold at which light intensity and spectrum become laserlike. Further advances will be necessary, however, to make a generally useful device.◀

Crown polymer extracts mercury from water

Removal and immobilization of mercury(II) ions from industrial waste streams is a difficult and expensive problem requiring a robust extractant that is resistant to corrosive waste. Chemists at Lawrence Livermore National Laboratory in Livermore, Calif., have developed an acid-resistant thiacrown polymer that has potential utility as a selective and cost-effective Hg^{2+} extractant. Postdoctoral associate Theodore F. Baumann and senior research chemists John G. Reynolds and Glenn A. Fox prepared a cross-linked polystyrene polymer containing pendant crown thioethers and showed that the polymer can quantitatively extract Hg^{2+} from aqueous media very rapidly [*Chem. Commun.,* **1998,** 1637]. The polymer was synthesized by copolymerization of a novel C-substituted crown thioether with divinylbenzene. The researchers were able to strip the bound Hg^{2+} ions from the polymer, allowing it to be reused without significant loss of loading capacity. "This thiacrown polymer is far more effective in extracting Hg^{2+} from aqueous solution than previously reported Hg^{2+} extractants," Baumann tells C&EN. The polymer selectively removes Hg^{2+} "even in the presence of a large excess of competing metal ions."◀



Experiments clarify O_2 formation on icy moons

New light is being shed on the process by which molecular oxygen could be formed on Jupiter's moons Ganymede and Europa. During the past few years, researchers have turned up evidence for the existence of condensed O_2 on Ganymede as well as for a tenuous oxygen atmosphere on Europa. Since both of these moons contain large amounts of water ice, it's thought that O_2 is formed when energetic particles from Jupiter's magnetosphere strike the ice. Researchers Thomas M. Orlando, Matthew T. Sieger, and William C. Simpson at Pacific Northwest National Laboratory in Richland, Wash., now have bombarded ice films chilled to 110 K with electron beams and have found that large amounts of O_2 are indeed produced [*Nature,* **394,** 554 (1998)]. They've also reevaluated the mechanism thought to be at work in this reaction. They found that the process originally believed to produce the O_2—a temperature-dependent mechanism in which radical precursors react to form O_2—doesn't fit their data. They developed a different model that involves a two-step mechanism. In the first step, an "incubation" dose of radiation produces stable precursor molecules, possibly HO_2 or H_2O_2. In the second step, another electronic excitation dissociates the precursor molecule to form O_2.◀

Simple polypeptides stick like mussel glue

Boat owners know how vexing mussels and barnacles can be: They stick to hulls like crazy. Their glues are paragons of moisture-resistant adhesives. For decades, researchers have been trying to mimic marine glues with synthetic versions for medical use. Previous efforts have focused on polypeptides containing L-dihydroxyphenylalanine (DOPA), L-lysine, L-tyrosine, or L-glutamic acid. Assistant professor of materials and chemistry Timothy J. Deming and graduate student Miaoer Yu at the University of California, Santa Barbara, now have developed systems for preparing large quantities of simple random copolymers of DOPA and L-lysine. In the presence of suitable oxidizing agents, aqueous solutions of the copolymers produce cross-linked networks that stick to various surfaces [*Macromolecules,* **31,** 4739 (1998)]. The work of Deming and Yu "is much more rigorous in the engineering and materials science aspects than previous work," says J. Herbert Waite, professor of marine biochemistry at the University of Delaware, Newark. The results unequivocally demonstrate the relationship of molecular weight and mole fraction of DOPA in the polymers, as well as the mode of oxidation, to changes in viscosity and tensile strength after oxidation, he adds. Thus by adjusting polymer composition, molecular weight, or oxidation conditions, adhesive properties can be tuned easily for specific applications.◀

technology concentrates

MS is rapid screen for enantiomeric excess

Two independent groups have devised high-throughput electrospray mass spectrometry (MS) techniques to measure enantiomeric excesses on micromole scales [*Angew. Chem. Int. Ed.*, **38**, 1755, 1758 (1999)]. The methods may find use in screening thousands of reaction mixtures per day in combinatorial chemical searches for enantioselective catalysts and reagents. The method reported by chemistry professor M. G. Finn, MS director Gary Siuzdak, and coworkers at Scripps Research Institute, La Jolla, Calif., involves the use of a reagent to label the products of a reaction. For example, they esterify a nonracemic mixture of chiral alcohols with a mixture of *N*-benzoyl-(*S*)-proline and *N*-*p*-tolyl-(*R*)-proline. The difference in reaction rates means that proline derivatives of different molecular weight acylate each alcohol enantiomer exclusively. The MS pattern reveals the amount of each alcohol enantiomer. The method of chemistry professor Manfred T. Reetz, MS department head Detlef Stöckigt, and coworkers at the Max Planck Institute for Coal Research, Mühlheim an der Ruhr, Germany, uses reaction substrates that are mass-labeled in the first place. To study enzyme-catalyzed esterification of α-phenylpropionic acid, for example, they use a 50-50 mixture of (*S*)-α-phenylpropionic and (*R*)-α-phenyl-β,β,β-d_3-propionic acids. The MS pattern of the differently mass-labeled ester enantiomers provides the relative amounts.◀

Behind the stickiness of mussel glue

For sticking to wet surfaces, the adhesive proteins of mussels are unbeatable, but exactly how they work has been a mystery. Scientists have speculated that L-3,4-dihydroxyphenylalanine (DOPA) and other amino acids are involved in the chemistry that leads to adhesion of the proteins and setting of the glue. Now, Timothy J. Deming, assistant professor of materials and chemistry at the University of California, Santa Barbara, and graduate students Miaoer Yu and Jungyeon Hwang have determined that DOPA is the only amino acid that's necessary for both adhesion and cross-linking of these proteins [*J. Am. Chem. Soc.*, published June 5 ASAP, http://pubs.acs.org/journals/jacsat]. Their work shows that the catechol (*o*-dihydroxybenzene) group in DOPA is responsible for adhesion through its ability to form very strong hydrogen bonds. "It can displace water molecules from water surfaces," Deming says. Some of the catechols are oxidized to *o*-quinones. When these quinones condense with each other, they form cross-links between different protein strands, setting the adhesive.◀

Another superfast folding protein

C&EN reported recently (May 31, page 28) on a study that found that prion protein folds in about 170 microseconds—a rate claimed to be faster than that for any known protein.

Now that record apparently has been toppled. Researchers at California Institute of Technology's Beckman Institute calculate that cytochrome b_{562}—a four-helix-bundle protein in which a heme group (red) is coordinated by two amino acid residues (yellow and green)—folds to its native state in only about 5 microseconds. Pernilla Wittung-Stafshede (now at Tulane University, New Orleans), Jennifer C. Lee, Jay R. Winkler, and Harry B. Gray calculate the rate in water by extrapolation of experimental measurements, using electron transfer as the folding trigger [*Proc. Natl. Acad. Sci. USA*, **96**, 6587 (1999)]. Cytochrome b_{562}'s record is not likely to be beaten by much because its folding rate is close to the theoretical "speed limit" for protein folding—about 1 microsecond for small single-domain proteins like cytochrome b_{562}.◀

Gene delivery teams up with tissue engineering

Biodegradable polymer matrices loaded with plasmid DNA have been shown to release their gene-bearing cargo slowly, leaking it out for up to a month [*Nat. Biotechnol.*, **17**, 551 (1999)]. The feat paves the way for sustained delivery of DNA fo gene therapy. But it also has application in tissue engineering because it provide a potential route to timing the delivery o genes involved at different stages of tis sue development. Lonnie D. Shea, Eliza beth Smiley, Jeffrey Bonadio, and Davi J. Mooney at the University of Michigan Ann Arbor, incorporated plasmid DN encoding β-galactosidase into a 3-D lacti acid-glycolic acid copolymer via a proces developed earlier by team leader Moo ney, a chemical engineer. They showe that the polymer slowly delivers plasmi DNA to cells growing in culture and tha the transfected cells express the encode enzyme. In animal studies, an implant o the copolymer loaded with a specifi growth factor enhanced blood vessel for mation in developing tissue. In an accom panying commentary, W. Mark Saltz man, a professor of chemical engineering at Cornell University, notes that sus tained DNA delivery could provide "new genes to replace those delivered previ ously that have ceased to function."◀

Catalytic antibody localizes drug activity

A new catalytic antibody-based method could direct drugs very specifically to target cells by catalyzing the removal of functional groups added in advance to block the drugs' activity. The method was devised at Scripps Research Institute, La Jolla, Calif., by President Richard A. Lerner, molecular biology professor Carlos F. Barbas III, and coworkers [*Proc. Natl. Acad. Sci. USA*, **96**, 6925 (1999)]. The catalytic antibody they used, 38C2, catalyzes hundreds of different aldol condensation reactions and was the first catalytic antibody to be marketed commercially. Now, the Scripps researchers use 38C2 for antibody-directed enzyme prodrug therapy with doxorubicin and camptothecin, anticancer drugs with serious side effects. By adding activity-masking groups to the drugs, they produce prodrug versions that are substantially less toxic. They show that the prodrugs are unmasked by 38C2 very selectively—with a reaction that is not known to be catalyzed by any other enzyme and that is very slow in the absence of 38C2. If a nonimmunogenic version of 38C2 can be targeted to tumors, it could activate prodrugs there in a very localized fashion, killing the cells—as demonstrated on colon and prostate cancer cell lines in the current study.◀

Outta sight! A crafty peek at the sun's back

When it comes to studying the far side of the sun, astronomers are no longer in the dark. Researchers reported last week that employing a detector on an orbiting spacecraft, they have had their first glimpse of the sun's hidden half.

Scientists may be able to use this new capability to provide earlier warnings of solar storms that will strike Earth, damaging satellites and disrupting power grids. Solar storms are expected to be on the rise as the sun reaches the maximum of its 11-year activity cycle next year.

The new discovery relies on detection of ultraviolet radiation emitted by the hydrogen gas that bathes our solar system. Radiation from the sun clears a bubble in this gas about the size of Earth's orbit, and the inside of the bubble can act like a giant theater screen. When energy emitted by a solar outburst strikes the screen, it produces ultraviolet hot spots.

An instrument aboard the SOHO (Solar and Heliospheric Observatory) spacecraft can detect these hot spots even from outbursts on the sun's hidden face. "We can monitor the back side of the sun without looking at it directly," says Jean-Loup Bertaux of the CNRS Service d'Aéronomie in Verrieres le Buisson, France. He presented the findings at a SOHO workshop in Paris.

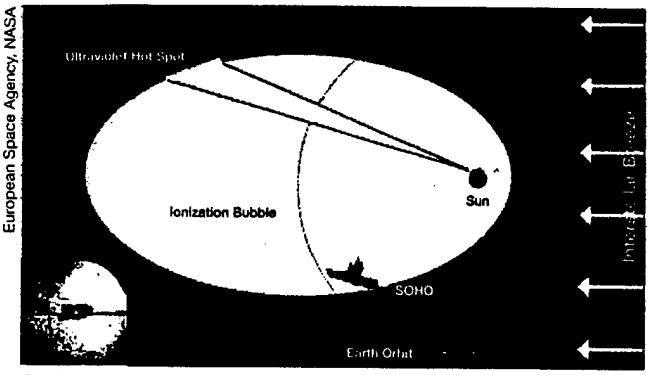
"Bertaux's work represents an entirely new way, and to my knowledge the only successful way, to identify the patterns of activity on the far side of the sun," says Craig DeForest of Stanford University.

Viewing hydrogen gas over the entire sky, a SOHO detector known as Solar Wind Anisotropies (SWAN) records a wavelength of ultraviolet radiation called Lyman alpha. Such light cannot be seen through Earth's atmosphere, but from SOHO's vantage point 1.5 million kilometers from our planet, it readily detects the radiation. When a solar storm erupts, the bubble of hydrogen gas radiates 5 to 15 percent more ultraviolet light than it normally does, says Bertaux.

Every solar disturbance gets carried along with the sun's 28-day rotation. As a result, each outburst is like a lighthouse beam, sweeping across the screen of hydrogen gas. Bertaux reports, "We have verified that the large areas of enhanced emission that we see are indeed moving on the sky with the 28-day period."

Bertaux told SCIENCE NEWS that his team has shown that storm activity on the sun's near side, imaged directly by another SOHO detector, also produces the expected ultraviolet glow recorded by SWAN. Moreover, when an active region on the near side rotates out of view, SWAN continues to detect an enhanced ultraviolet glow from the hydrogen gas.

The technique has also uncovered disturbances that originate on the farside and then rotate into Earth's view. Monitoring the sky at the Lyman-alpha wavelength "therefore offers a unique opportunity to detect in advance some new solar activity," Bertaux notes.



Lightest region indicates storm activity on the farside, recorded July 20, 1996. The sun's radiation carves a bubble in the hydrogen gas in which the solar system is embedded. Ultraviolet light from a solar eruption strikes the inside of the bubble's surface, generating a hot spot. Inset: Celestial hemisphere illuminated by ultraviolet radiation from the sun's farside.

ly a research tool, it has the potential to give us a much longer lead time," as much as 14 days, for predicting solar storms, agrees Ron D. Zwickl of the National Oceanic and Atmospheric Administration's Space Environment Center in Boulder, Colo.

Richard C. Canfield of Montana State University in Bozeman points out that the method cannot distinguish outbursts likely to pose a threat to Earth from those that may turn out to be weaklings. Estimating the power of outbursts requires other diagnostic tools now available only for the sun's near side. These include direct images of twisted magnetic fields in the sun's atmosphere, Canfield says (SN: 3/13/99, p. 164). *—R. Cowen*

Amino acid puts the muscle in mussel glue

The proteins that mussels use to anchor themselves underwater derive their strength and stickiness from a single amino acid, according to a new study. The finding could help researchers develop moisture-resistant glues for biomedical and industrial purposes.

The modified amino acid, called dihydroxyphenylalanine (DOPA), has two functions. It allows the proteins containing it to stick to a variety of surfaces and also to set into a tough, rubbery cement, report Miaoer Yu, Jungyeon Hwang, and Timothy J. Deming of the University of California, Santa Barbara (UCSB).

"The fact that they have identified one [compound] doing those two completely different things is quite intriguing to me," says Herbert Waite, a marine biochemist at UCSB not connected with the current study. "In industry, people generally use different reagents for those two functions."

Mussels, before they end up on a seafood lover's plate, use fibers known as byssal threads to tether themselves (SN: 1/5/91, p. 8). Flat adhesive plaques at the ends of the threads bind to surfaces, allowing the bivalves to hang on even while buffeted by strong waves.

A blue mussel (Mytilus edulis) *can anchor itself underwater with strong, sticky proteins.*

To make the glue, mussels first connect standard amino acids into chains and then modify them chemically, says Waite. DOPA results when an enzyme adds a second hydroxyl group to the amino acid tyrosine. Though not the most abundant amino acid in the adhesive, DOPA makes up 5 to 20 percent of its content.

To investigate DOPA's role, Deming's group synthesized model proteins containing 5 percent DOPA and the rest glutamic acid. These molecules bound much more strongly to aluminum than did proteins containing only glutamic acid.

The researchers also discovered that DOPA, in an oxidized form, is responsible for cross-linking protein strands. "That cross-linking has to kick in in order for that adhesive to perform," says Waite. "Otherwise, it's only bound to the surface, not to itself." Deming and his colleagues report their findings in the June 23 JOURNAL OF THE AMERICAN CHEMICAL SOCIETY.

Unlike industrial adhesives used today, such protein-based glues would be made using water instead of harsh organic solvents. Also, the ability of the mussel adhesive to stick and set in water makes it attractive for medical and dental applications. In animals and laboratory cultures of human cells, the mussel adhesive does not appear to be toxic, Waite notes. Only further studies will reveal how DOPA makes the protein waterproof. *—C. Wu*